FINAL REPORT FOR GRANT #NGR 15-003-053

Control Mechanisms in Physiological Rhythms

Our grant was concerned with elucidating the factors which are involved in regulating rhythmic phenomena. We attacked this problem from essentially one basic premise. This was, that at a particular point in time, any cell normally can act in only one of two ways. It can be engaged in either dividing or in performing its particular function; a normal cell's energies are usually directed in one or the other of these tasks by means of its basic control mechanisms.

Our working hypothesis was that if we could identify rhythms of cell division and of function and determine the mechanisms by which we could alter these rhythms we might find: 1) that the rhythms of division and function were inversely related and, 2) that the control mechanism were basically similar.

Because of the necessity of utilizing large numbers of animals we chose Rana pipiens as our test animal. The frog is small, easily stored and handled, relatively inexpensive, and available in large numbers. We have used literally thousands of these vertebrates.

We chose DNA content and synthesis of dorsal epidermis and corneal epithelium and mitotic activity of dorsal epiermis as representing cell division. To represent cell function, we chose plasma corticosterone levels.

We had previously found that corneal epithelium exhibited a rhythm in mitotic division. Present studies conducted on DNA synthesis showed that DNA exhibited a rhythm with a period of approximately 24 hours which correlated well with previous mitotic division rhythms. Our studies also indicated that the rhythm of DNA synthesis in corneal epithelia seemed to be regulated by the lighting regime to which the animals were exposed (2,6).

We also investigated rhythms in dorsal epidermis. Both DNA content and DNA synthesis showed diurnal variations which were reproducible and had periodicities of approximately 24 hours. We also have indications that these rhythms are regulated by changes in the lighting regime (1,5).

Statistically significant rhythms of mitotic division in the same tissue were also observed. These rhythms were reproducible and had periodicities of 18-24 hours depending on the sex of the animal. Although inverting the lighting regime for 14 days was not sufficient to completely invert the rhythm of cell division, it appears that the lighting regime is important in this phenomena. Animals which were either blinded or parapinealectomized (frontal organ or parapineal body of the pineal system cauterized) and tested after 14 days in controlled conditions showed no evidence of rhythms in cell division (3).

As a tangential problem we were able to use the same animals to test the role of changes in environment as a possible stimulus for inducing the development of kidney tumors. These studies showed that there was an increased incidence of Lucké adenocarcinoma as seen from a histological study than had been observed previously from a gross point of view (4 ). Preliminary evidence also indicated that this was due to stress of a seasonal nature.

We have also investigated the effect of constant light and constant dark conditions on mitotic division rhythms (14).

We were able to observe reproducible rhythms of plasma corticosterone (7, 10). These rhythms are synchronized by the lighting regime and appear to be regulated through the parapineal-pineal system (7, 13). We were also able to observe reproducible rhythms in eosinophils (8,11) as well as seasonal changes in corticosterone level and hematocrit and eosinophil levels (8, 12).

There seems to be an inverse correlation between the rhythms of cell division and cell function that we have studied. Apparently the lighting regime is important in regulating these rhythms. The pineal organ system appears to be involved in our test animal in the perception of the light stimulus. However, this has to be studied in more detail. We hope our future work will be able to more clearly characterize these phenomena and any correlation between them.

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REPORTS SUPPORTED BY NGR 15-003-053

A. Papers

1. Morgan, W.W. and S. Mizell, Diurnal fluctuations in DNA content and DNA synthesis in the dorsal epidermis of Rana pipiens, Comp. Biochem. and Physiol. 38:591-602 (1971).

2. Morgan, W.W. and S. Mizell, Daily fluctuations of DNA synthesis in the corneas of Rana pipiens, Comp. Biochem. and Physiol. 40:487-493 (1971).

3. Garcia-Arce, H. and S. Mizell, Mitotic activity in dorsal epidermis of Rana pipiens. Comp. Biochem. & Physiol. 42:501-510 (1972).

4. Marlow, P.B. and S. Mizell, Incidence of Lucké Renal Adenocarcinoma in Rana pipiens as determined by histological examination, J. Nat. Cancer Instit., 48:823-829 (1972).

B. Abstracts

5. Morgan, W.W. and S. Mizell, Rhythmic fluctuations in DNA synthesis and DNA content in tissues of Rana pipiens, Fed. Proc. 29:837 (1970).

6. Morgan, W.W. and S. Mizell, Daily and seasonal fluctuations in corneal DNA synthesis of adult Rana pipiens, Physiol., 13:264 (1970).

7. Akin, D.P. and S. Mizell, Control mechanisms in rhythms of plasma corticosterone, Fed. Proc. 30:610 (1971).

8. Akin, D.P. and S. Mizell, Seasonal changes in hematocrit and eosinophil and in plasma corticosterone level. Anat. Rec. 169:266 (1971).

9. Mizell, S. and D.P. Akin, Daily and seasonal rhythms of plasma corticosterone, Proc. Intern. Union of Physiol. Sci. 9:397 (1971).

C. In Preparation

10. Mizell, S. and D.P. Akin, Rhythms of plasma corticosterone in Rana pipiens.

11. Mizell, S. and D.P. Akin, Short period rhythms of eosinophil level in Rana pipiens.

12. Mizell, S. and D.P. Akin, Seasonal rhythm of plasma corticosterone, hematocrit and eosinophil level in Rana pipiens.

13. Mizell, S. and D.P. Akin, The role of light and the pineal gland in regulating plasma corticosterone rhythms.

14. Mizell, S. and H. Garcia-Arce, The effect of constant lighting conditions on mitotic division rhythms.